Fate of unirradiated *Salmonella* in irradiated mechanically deboned chicken meat *

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Mechanically deboned chicken meat was irradiated at 0, 1.25 and 2.50 kGy (Cesium 137) and inoculated with *Salmonella dublin* ATCC 15480, *Salmonella enteritidis* ATCC 9186 or *Salmonella typhimurium* ATCC 14028. Samples were then stored at 5°C and 10°C and were subjected to microbiological analysis directly after irradiation and inoculation (time 0), and after 24, 72, 120, 168 and 216 h of storage. Samples stored at 20°C were examined at time 0 and after 6, 12 and 24 h of storage. Irradiation at 1.25 and 2.50 kGy caused an average reduction in bacterial levels of 2.23 and 3.44 logs, respectively. *S. dublin, S. enteritidis* and *S. typhimurium* showed very small, insignificant changes in numbers, during storage of meat for 9 days at 5°C. The final populations of *S. dublin* and *S. typhimurium* in samples irradiated before inoculation and stored at 10°C or 20°C were greater than the equivalent populations in samples which had not been irradiated before inoculation. Reduction of indigenous microflora in mechanically deboned chicken meat by irradiation may create better conditions for the growth of salmonellae and may thus increase the risk of salmonellosis when accidental contamination and temperature abuse occur after a radiation treatment. Therefore, irradiated mechanically deboned chicken meat should be properly refrigerated and protected against contamination.

Introduction

Epidemiological studies conducted at many locations have shown that 30 to 50% of chicken carcasses are contaminated with *Salmonella* (Silliker, 1982; Rudy, 1986; Szczawińska, 1989), and human salmonellosis is frequently associated with consumption of poultry products (Todd, 1980; Bryan, 1981). One of the main reasons for the above is symptomless carrying of salmonellae by animals destined for slaughter.

Kampelmacher (1981) concluded that the proper use of ionizing radiation seems to be the most promising and effective way to reduce the incidence of *Salmonella* contamination of poultry. Pre-packing of poultry after slaughter and irradiation with a dose of 2–5 kGy effectively eliminated salmonellae (Mulder, 1977; Doelstaedt et al., 1990). Radiation treatment seems to be particularly useful for decontamination of mechanically deboned poultry meat (Thayer et al., 1990). This comminuted product obtained from poultry parts (necks, backs and racks), which were not utilized previously (Froning, 1976), has recently been given special attention and created a demanding market. Mechanically deboned poultry meat is often heavily contaminated both with spoilage and pathogenic bacteria, including salmonellae (Ostovar et al., 1971). It may create economic and sanitary problems, because the product is frequently shipped long distances to the final point of processing. At present the treatment of mechanically deboned poultry meat with ionizing radiation to eliminate non-spore-forming pathogens is technically feasible and applied on a relatively wide scale in France (Gallien et al., 1983, 1985; Roussel, 1988).

Treatment of poultry meat with ionizing radiation doses of 1 to 10 kGy produces major changes in the microflora. Most vegetative bacteria, including pathogens, are eliminated and the post-irradiated microflora is different in composition from the pre-irradiated one (Ingram and Farkas, 1977; Maxcy, 1983). In most cases, the spoilage microflora of red meat, fish and poultry held at refrigeration temperatures consisted primarily of Gram-negative psychrotrophs, whereas after irradiation the flora was predominantly Gram-positive cocci and bacilli (Prachasitthisakdi et al., 1984; Dickson and Maxcy, 1985). The radiation-induced reduction and shift in spoilage microflora may create better conditions for the growth of some pathogenic bacteria by elimination of competitive microorganisms. Higher growth rates for staphylococci, *C. botulinum* and other bacteria on pasteurized or sterilized products than on raw meats were reported by some authors (Pliszka, 1979; Szczawiński, 1987).

The purpose of this study was to compare the survival or multiplication of unirradiated salmonellae in unirradiated or irradiated mechanically deboned chicken meat inoculated after radiation treatment and incubated at refrigeration temperature (5°C) and under temperature abuse (10°C and 20°C). Another way of stating the purpose is as follows: is there a greater risk from the same number of *Salmonella* cells contaminating irradiated or unirradiated meat.

Materials and Methods

Preparation of meat samples

Mechanically deboned chicken meat was obtained from a commercial manufacturer of poultry frankfurters and consisted of approximately 90% rib meat and 10% back meat. The proximate analysis of this product was 64.77% moisture, 18.97% fat, 15.00% protein, and 1.14% ash (analysis performed by QC Inc., Southampton, PA). The chicken meat was subdivided into 100-g lots and vacuum

sealed in Stomacher® 400 (Tekmar, Co., Cincinnati, OH) polyethylene bags, which were in turn vacuum-sealed in American Can Company Freshstuff® (American Can Company, Greenwich, CT) bags. The oxygen and water transmission rates for the Freshstuff® bag are 0.6–0.8 $cm^3/645.2\ cm^2/24$ h at 22.8°C and 50% R.H., and 0.3–0.4 $g/645.2\ cm^2/24$ h at 37.8°C and 90% R.H., respectively. These samples were then stored at −20°C until used.

Immediately before use, the meat was thawed in a water bath at 30°C and mixed. The meat was aseptically divided into 4.5 ± 0.05 g samples and then packed aerobically in Stomacher® 400 polyethylene bags.

Irradiation

Samples were irradiated at 0°C at 0, 1.25 and 2.50 kGy in a self-contained cesium 137 gamma radiation source of 135 000 Ci and a dose rate of 0.12 kGy per min. The dosimetry and dose distribution for this radiation source were as described by Shieh et al. (1985). Routine dosimetry was conducted with ferrous sulfate/cupric sulfate dosimeters (IAEA, 1977).

Test organisms and inoculation of meat samples

Salmonella dublin ATCC 15480, *Salmonella enteritidis* ATCC 9186 and *Salmonella typhimurium* ATCC 14028 were used in these studies.

Each strain was maintained and transferred on Tryptic soy agar (TSA) (Difco, Detroit, MI) with incubation at 35°C. Culture purity and identity were verified using API 20 E diagnostic kits (API Analytab Product, Plainview, NY) and *Salmonella* diagnostic sera (Fisher Diagnostics). The bacteria were transferred into Trypticase soy broth (Difco, Detroit, MI USA) and incubated 18 h at 35°C. The inoculum was prepared by diluting the broth in sterile 0.1% peptone water to obtain a suspension containing approx. 1×10^4 viable salmonellae/ml. Samples were inoculated within 1 h after irradiation by adding 0.5 ml bacterial suspension into 4.5 g of meat. After inoculation the samples were kneaded to obtain a uniform distribution of the bacteria in the meat.

Storage of meat samples

Immediately after irradiation and inoculation, meat samples were placed in incubators and stored for 9 days at 5°C and 10°C, and for 24 h at 20°C.

Microbiological analysis

Samples stored at 5°C and 10°C were analysed microbiologically immediately after irradiation and inoculation (time 0), and after 24, 72, 120, 168 and 216 h of storage. Samples stored at 20°C were examined at time 0 and after 6, 12 and 24 h of storage. Samples were homogenized in a Stomacher® 400 in appropriate volumes of sterile 0.1% peptone water.

Salmonellae were enumerated using brilliant green sulfa agar (Difco, Detroit, MI) or Brilliant Green Agar (BBL, Cockeysville, MD) with the addition of 1.0 g sodium sulfapyridine (Sigma Chemical Co., St. Louis, Missouri). Plates were incubated for 24 h at 35°C. The salmonellae were verified using bismuth sulfite

agar (Difco, Detroit, MI) and API 20 E diagnostic kits. Finally the colonies suspected to be *Salmonella* were confirmed serologically with O antisera.

Aerobic plate counts (including salmonellae) were determined on Tryptic soy agar (BBL), incubated for 48 h at 35°C. Colonies on TSA were counted using an automated colony counter Biotran II (New Brunswick Scientific Co., Inc., Edison, NJ). Surface plating was done using three plates for each dilution.

Statistical analysis

The experiments were repeated three times for the samples stored at 5°C and four times for the samples stored at 10°C and 20°C. The average of the colony counts (cfu) for the three plates was obtained for each replicate sample.

The bacterial counts were transformed to $\log_{10}$. The transformed data were analysed by using a Statistical Analysis System (SAS Institute Inc., 1987) computer program with a general linear model procedure. Comparison of means was based on Tukey's multiple range test (Miller, 1981; Wójcik et al., 1984; Walewski, 1989).

Results and Discussion

Effect of irradiation on aerobic bacterial counts in mechanically deboned chicken meat prior to inoculation with salmonellae

Initial contamination of chicken meat ranged from 7.59×10^4 to 9.55×10^5 colony forming units (cfu/g) with an average of 2.84×10^5, i.e., 5.54 log units (Table I). The lowest aerobic plate counts (APC) were found in meat samples used in the experiments with *S. dublin*. The average contamination was 1.49×10^5

TABLE I

Reduction of indigenous microflora by ionizing radiation treatment of mechanically deboned chicken meat

Samples of meat prepared for experiment, before inoculation with	Dose (kGy)	Aerobic plate count (log) ± SD [a] in Samples prepared for storage at		
		5°C ($n = 3$)	10°C ($n = 4$)	20°C ($n = 4$)
S. dublin	0.00	5.70 ± 0.01	4.94 ± 0.21	4.88 ± 0.05
	1.25	3.44 ± 0.09	2.74 ± 0.48	3.12 ± 0.25
	2.50	2.17 ± 0.14	1.77 ± 0.64	2.11 ± 0.16
S. enteritidis	0.00	5.98 ± 0.54	5.73 ± 0.66	5.80 ± 0.52
	1.25	3.48 ± 0.47	3.40 ± 0.41	3.31 ± 0.25
	2.50	2.22 ± 0.04	2.18 ± 0.09	2.04 ± 0.19
S. typhimurium	0.00	5.57 ± 0.17	5.36 ± 0.21	5.12 ± 0.24
	1.25	3.36 ± 0.04	2.75 ± 0.85	3.41 ± 0.21
	2.50	1.91 ± 0.16	1.59 ± 0.57	2.09 ± 0.10

[a] cfu/g of meat at time zero.

cfu/g. The highest APC were obtained for the samples of meat prior to inoculation with *S. enteritidis* (the average contamination of all samples was 6.87×10^5 cfu/g). Irradiation at a dose of 1.25 kGy caused a 1.71 to 2.6 log reduction in numbers with an average of 2.23 logs (Table I). A dose of 2.5 kGy reduced APCs by 2.77 to 3.77 logs with an average of 3.44 logs. Similar APC reduction by ionizing radiation in whole chicken carcasses (Basker et al., 1986), minced (Szczawińska et al., 1982) or mechanically recovered poultry meat (Gallien et al., 1983) and raw red meats (Tiwari and Maxcy, 1971) was observed in previous studies.

Effect of irradiation on behavior of salmonellae and changes in aerobic plate counts in mechanically deboned chicken meat during storage at 5 ° C

None of the three tested *Salmonella* strains proved capable of growth at 5°C, as expected (Figs. 1, 2, 3). Similar results were reported by Tiwari and Maxcy (1972). The numbers of salmonellae decreased slightly during storage in all inoculated samples; however, this decrease was statistically significant ($P < 0.01$) only in samples inoculated with *S. enteritidis* (Table II). The differences between control and irradiated samples were not significant by Tukey's test. In contrast to the populations of salmonellae, the populations of other microflora increased at 5°C during storage (Figs. 4, 5, 6). The analyses of variance indicated that storage time as well as irradiation exerted a statistically significant effect on APC in all three experiments (Table II). Significant interactions of storage time with radiation dose in experiments with *S. dublin* and *S. typhimurium* (Table II) suggest that irradiation not only caused an initial reduction of indigenous bacteria but also may have significantly altered their multiplication during storage or selected for species and/or genera with slower rates of growth (Table II). In most cases irradiated

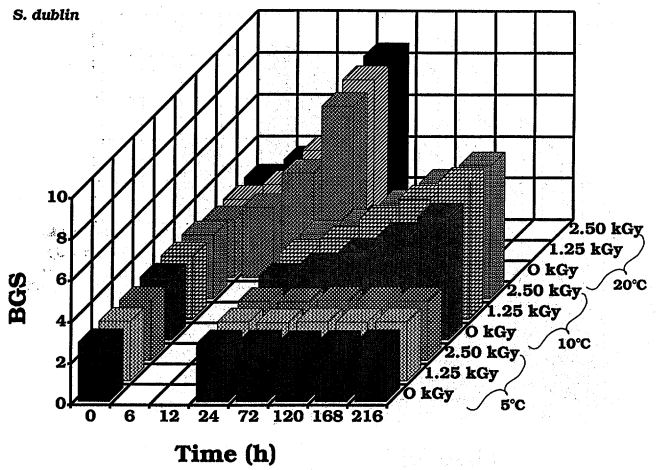

Fig. 1. Behavior of *S. dublin* when inoculated into unirradiated and irradiated (1.25 and 2.50 kGy) mechanically deboned chicken meat at storage temperatures of 5, 10 and 20°C. BGS, logarithm (base 10) of number of *S. dublin* cfu/g of meat determined on Brilliant Green sulfa agar. Time (h) indicates the number of hours of incubation at the temperature indicated.

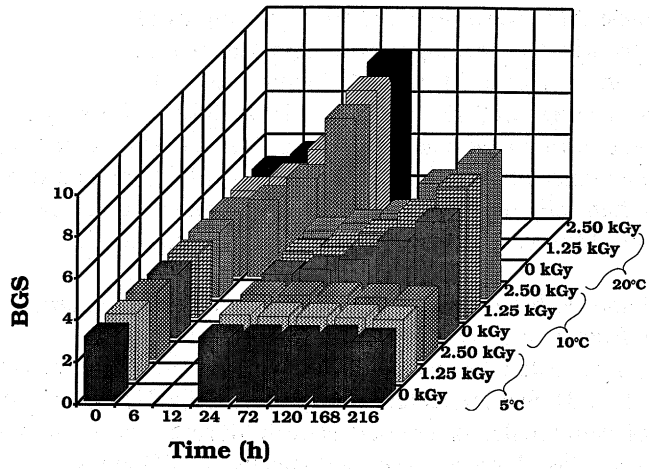

Fig. 2. Behavior of *S. enteritidis* when inoculated into unirradiated and irradiated (1.25 and 2.50 kGy) mechanically deboned chicken meat at storage temperatures of 5, 10 and 20°C. BGS, logarithm (base 10) of number of *S. enteritidis* cfu/g of meat determined on Brilliant Green sulfa agar. Time (h) indicates the number of hours of incubation at the temperature indicated.

indigenous microflora had dose-related increased lag phases and decreased rates of multiplication compared with that of the indigenous microflora in the unirradiated control (Figs. 4, 5, 6). As a result initial differences in APC between control and irradiated samples increased systematically with storage time. Such behavior

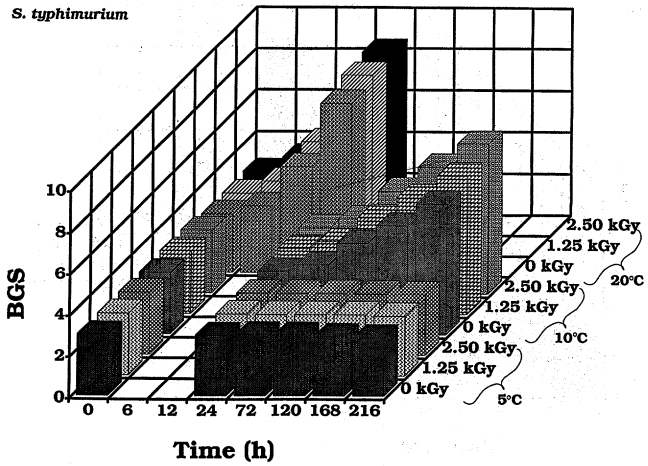

Fig. 3. Behavior of *S. typhimurium* when inoculated into unirradiated and irradiated (1.25 and 2.50 kGy) mechanically deboned chicken meat at storage temperatures of 5, 10 and 20°C. BGS, logarithm (base 10) of number of *S. typhimurium* cfu/g of meat determined on Brilliant Green sulfa agar. Time (h) indicates the number of hours of incubation at the temperature indicated.

TABLE II

F values in analysis of variance for microbial counts

Temperature	Microorganism	Medium	Main effect		
			Storage time	Radiation dose	Interaction time × dose
5°C	*S. dublin*	BGS	1.03	5.04 *	0.31
	S. enteritidis	BGS	12.99 **	6.56 **	0.65
	S. typhimurium	BGS	1.11	1.87	1.18
	S. dublin	TSA	42.47 **	623.17 **	9.99 **
	S. enteritidis	TSA	12.86 **	119.18 **	1.07
	S. typhimurium	TSA	57.24 **	740.61 **	14.02 **
10°C	*S. dublin*	BGS	435.51 **	24.01 **	2.91 *
	S. enteritidis	BGS	39.39 **	1.55	0.27
	S. typhimurium	BGS	210.00 **	8.78 **	0.95
	S. dublin	TSA	257.78 **	458.45 **	8.53 **
	S. enteritidis	TSA	244.54 **	413.81 **	6.86 **
	S. typhimurium	TSA	132.22 **	258.41 **	3.47 **
20°C	*S. dublin*	BGS	4565.30 **	14.93 **	1.70
	S. enteritidis	BGS	74.75 **	0.48	0.13
	S. typhimurium	BGS	9168.28 **	47.59 **	4.91 **
	S. dublin	TSA	4120.87 **	716.90 **	42.07 **
	S. enteritidis	TSA	163.19 **	77.31 **	1.93
	S. typhimurium	TSA	1235.88 **	286.52 **	18.90 **

* Significance at $P < 0.05$.
** Significance at $P < 0.01$.

of irradiated bacteria in meat was reported by others (Idziak and Incze, 1968; Tiwari and Maxcy, 1972; Farkas, 1987).

Effect of irradiation on behavior of salmonellae and changes in aerobic plate counts in mechanically deboned chicken meat during storage at 10°C

As shown in Fig. 1, 2, and 3, all tested *Salmonella* strains multiplied during storage of the meat samples at 10°C. Analysis of variance (Table II) as well as Tukey's test indicated that the populations of *S. dublin* were lower in control samples after 120, 168, or 216 h incubation at 10°C than in meat that had received a radiation dose of 1.25 kGy before inoculation. The populations of *S. dublin* in meats irradiated with 1.25 and 2.50 kGy were not significantly different (Fig. 1). Mean values presented in Fig. 1, 2, and 3 suggest that a general pattern of growth of *S. enteritidis* in control and irradiated meat samples was similar to that observed in experiments with *S. dublin*. However, the differences between unirradiated and irradiated samples inoculated with *S. enteritidis* were not statistically significant. Significant effects of storage time and radiation dose found in the analysis of variance for *S. typhimurium* counts suggest that growth of this microorganism was

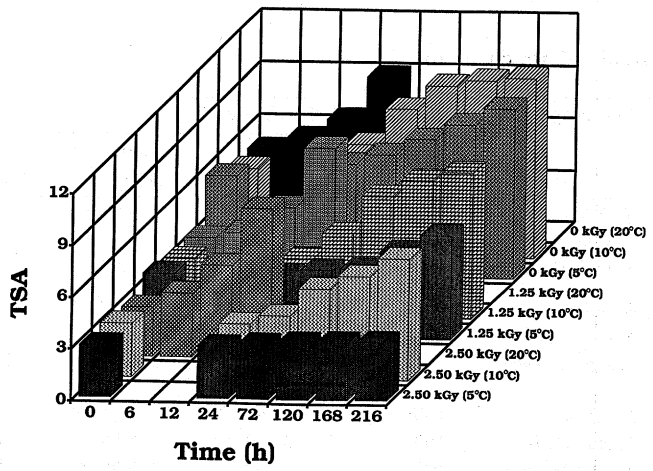

Fig. 4. Total aerobic plate count on tryptic soy agar for *S. dublin* and indigenous microflora when the *S. dublin* was inoculated into unirradiated and irradiated (1.25 and 2.50 kGy) mechanically deboned chicken meat and stored at temperatures of 5, 10 and 20°C. TSA, logarithm (base 10) of cfu/g of meat of *S. dublin* and indigenous microflora assayed on tryptic soy agar. Time (h) indicates the number of hours of incubation at the temperature indicated.

more rapid in irradiated than in non-irradiated meat samples (Table II); however, the differences between control and irradiated samples observed after particular periods of storage were not significant when mean values were compared with Tukey's test.

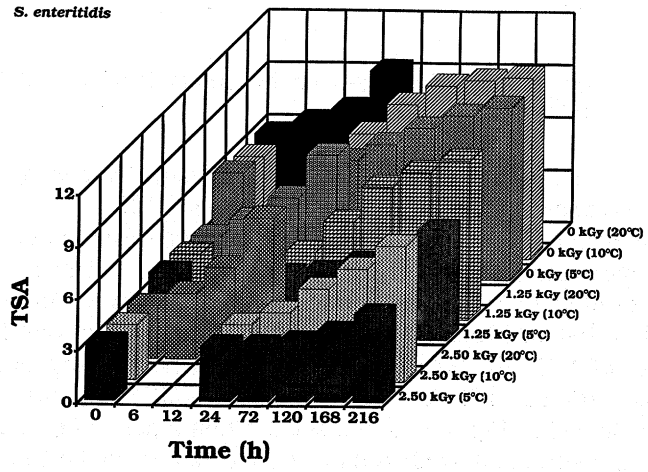

Fig. 5. Total aerobic plate count on tryptic soy agar for *S. enteritidis* and indigenous microflora when the *S. enteritidis* was inoculated into unirradiated and irradiated (1.25 and 2.50 kGy) mechanically deboned chicken meat and stored at temperatures of 5, 10 and 20°C. TSA, logarithm (base 10) of cfu/g of meat of *S. enteritidis* and indigenous microflora assayed on tryptic soy agar. Time (h) indicates the number of hours of incubation at the temperature indicated.

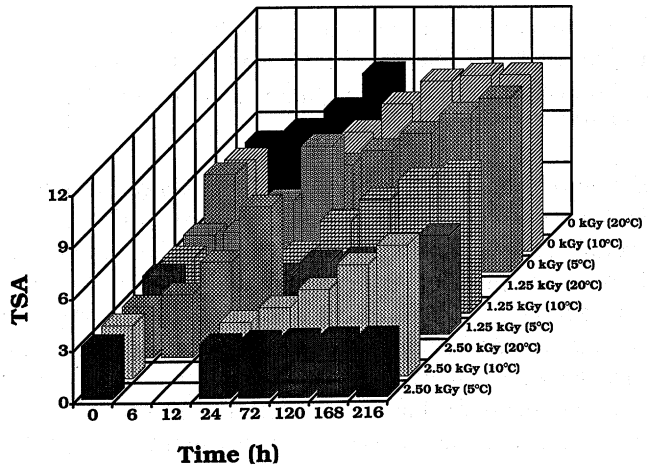

Fig. 6. Total aerobic plate count on tryptic soy agar for *S. typhimurium* and indigenous microflora when the *S. typhimurium* was inoculated into unirradiated and irradiated (1.25 and 2.50 kGy) mechanically deboned chicken meat and stored at temperatures of 5, 10 and 20°C. TSA, logarithm (base 10) of cfu/g of meat of *S. typhimurium* and indigenous microflora assayed on tryptic soy agar. Time (h) indicates the number of hours of incubation at the temperature indicated.

The changes in APC (indigenous microflora plus salmonellae) were similar in all experiments irrespective of the *Salmonella* strain used for inoculation (Figs. 4, 5, 6 and Table II), but the indigenous bacteria increased more rapidly during storage than the number of salmonellae in both the control and irradiated samples. The increase of APC was the most dynamic in unirradiated meat samples, less intensive in samples irradiated with 1.25 kGy, and the slowest in samples irradiated with a dose of 2.5 kGy where domination of salmonellae over natural contaminants was the most evident (compare Fig. 1 with Fig. 4; Fig. 2 with Fig. 5 and Fig. 3 with Fig. 6).

Considering the results presented in Figs. 1, 2, and 3, it is of interest to note that the salmonellae populations were lowest in unirradiated meat samples in which the highest indigenous microbial populations occurred. Irradiation of meat at 1.25 and 2.50 kGy prior to inoculation caused a dose-dependent reduction of inherent microflora and was associated with larger populations of *Salmonella* following incubation at 10°C.

Interactions of mixed bacterial populations may be synergistic or antagonistic. Antagonistic processes include direct predation or parasitism among organisms and different forms of competition for space and nutrients, such as impoverishing the environment leading to inhibition of growth of other microorganisms, changes in pH and E_h, or formation of antimicrobial products (Sinell, 1980). Irrespective of the mechanism by which the natural microflora retard the growth of salmonellae, this seems to have occurred under the conditions of these experiments. However, it should be emphasized that the effect of the indigenous microbial flora of mechanically deboned chicken meat on salmonellae was rather slight and the relatively

strong reduction of this microflora by irradiation did not always significantly affect the growth of *Salmonella* (Table II). In addition, the differences in *Salmonella* counts between control and irradiated samples were in most cases not statistically significant ($P > 0.01$).

Effect of irradiation on behavior of salmonellae and changes in aerobic plate counts in mechanically deboned chicken meat during storage at 20°C

Salmonella counts rapidly increased at 20°C in all experiments (Figs. 1, 2, and 3), and after 24 h of storage salmonellae reached a higher population density than after 216 h of storage at 10°C. After each period of storage, the population of *S. typhimurium* in meat that had been irradiated at 2.5 kGy was always higher than in unirradiated ones. However, analyses of variances (Table II) as well as Tukey's test indicated that the numbers of salmonellae were statistically affected by irradiation of the samples before inoculation only in experiments with *S. dublin* and *S. typhimurium*.

The changes in APC (indigenous microflora plus *Salmonella*) in experiments with *S. dublin* and *S. typhimurium* were almost identical. In both cases, storage time, irradiation, and interaction of both of these factors were statistically significant in analysis of variances. In experiments with *S. enteritidis*, the results were similar, except that the interaction between storage time and irradiation was not significant.

In contrast to the results obtained at storage temperatures of 5°C and 10°C (Figs. 1, 2, and 3), the differences between the numbers of *Salmonella* and APC of unirradiated samples stored at 20°C, were very small. This suggests that the microflora of mechanically deboned chicken meat stored at 20°C was strongly dominated by the *Salmonella*. In irradiated samples, the number of salmonellae and APC after particular periods of storage were almost equal (Figs. 1, 2, and 3).

Though the population of the indigenous microflora increased in unirradiated mechanically deboned chicken meat more slowly than did the *Salmonella* during storage at 20°C, and their final numbers were lower than in meats stored at 10°C, those indigenous populations exerted a statistically significant, antagonistic influence on two out of the three *Salmonella* strains tested.

The rapid growth of salmonellae in unirradiated meats stored at 20°C is well known and the results obtained for unirradiated samples are in agreement with other studies (Szczawiński et al., 1985). It is difficult to estimate whether the elimination of the competitive microflora by irradiation may increase the risk of salmonellosis from irradiated mechanically deboned chicken meat to the extent which could be important from a practical point of view. Further, though significant effects of radiation dose were observed, it should be noted that the challenge inocula of approximately 1000 cfu of salmonellae per gram of meat would be a considerably larger population than would be expected on properly handled products. Poultry meat is often contaminated with salmonellae (Kałużewski et al., 1988), and the hazard of salmonellosis from unirradiated mechanically deboned chicken meat stored at 10°C seems to be much higher than the hazard related to possible recontamination of that meat with *Salmonella* after irradiation. The

results confirm the opinion that proper refrigeration is an important factor in the prevention of salmonellosis and that it cannot be replaced by low to medium dose treatments with ionizing radiation. The behavior of salmonellae surviving radiation treatment in meat during storage under temperature abuse has been reported by some authors (Szczawińska et al., 1982; Tiwari and Maxcy, 1972). However, information on the growth of unirradiated salmonellae in control and irradiated mechanically deboned chicken meat has not been found in the literature.

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